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TIMED ARTIFICIAL INSEMINATION (AI) IN CATTLE: IMPROVING USE AND PREGNANCY RATES

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TIMED ARTIFICIAL INSEMINATION (AI) IN CATTLE: IMPROVING USE AND PREGNANCY RATES

Reuben J. Mapletoft and John P. Kastelic*

WCVM, University of Saskatchewan

*AAFC, Lethbridge Research Centre

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Abstract/Summary

The overall objective was to refine protocols, developed largely by ourselves, that synchronize ovulation, resulting in high fertility to timed-AI (TAI; i.e. AI by appointment, without estrus detection). Specific objectives were to simplify protocols, use commercial products, and improve pregnancy rates to TAI by increasing the proportion of cattle responding to follicle synchronizing treatments and promoting growth and maturation of the preovulatory follicle.

We previously reported that estradiol and progesterone synchronized follicle wave emergence in a two-injection prostaglandin (PGF) treatment protocol for TAI. That some cattle ovulated before TAI suggested that the estradiol caused premature regression of the corpus luteum (CL). In a detailed ultrasound study, estradiol benzoate given 7 d after a single injection of PGF effectively synchronized follicular development, although concurrent progesterone treatment appeared to suppress CL function. Therefore, progesterone and not estradiol may have caused premature estrus in previous studies.

Although MGA was used as a progestin source in confined cattle, it consistently yielded lower pregnancy rates to TAI than progesterone-based protocols (perhaps due to variations in absorption and metabolism). In contrast, a 7- or 8-day CIDR protocol was very effective. Although presynchronization with PGF (with or without a used CIDR) increased the proportion of cattle responding to the first injection of GnRH or pLH in a GnRH-PGF-GnRH protocol for TAI, effects on pregnancy rates were variable and inconclusive. The addition of a progesterone-releasing vaginal insert to a GnRH-based protocol increased pregnancy rates in heifers, but not in lactating beef cows. Presynchronization was without benefit in estradiol-CIDR based protocols. Giving eCG at PGF treatment increased pregnancy rates to TAI in younger cows (that were also in poorer body condition and less likely to be cycling), whether they were synchronized with GnRH or estradiol, but was without benefit in heifers. Furthermore, eCG at the predicted time of follicle wave emergence in recipient cattle increased pregnancy rates to timed-embryo transfer.

Although treatments after TAI to increase progesterone concentrations did not increase pregnancy rates to TAI or embryo transfer, progestin treatment after TAI synchronized returns to estrus. The CL forming from a persistent follicle was as efficacious as that from normal preovulatory follicle in supporting pregnancy in embryo transfer recipients, but did not produce more progesterone. The addition of a CIDR or eCG to a GnRH-based synchronization protocol yielded no improvement in recipient selection rates, or pregnancy rate following fixed-time embryo transfer.

In several experiments to determine why heifers were less responsive than lactating dairy cows to GnRH treatment, elevated progesterone concentrations suppressed GnRH-induced LH release and ovulation. However, there was no difference between heifers and lactating beef cows; the latter appeared to be more similar to heifers than lactating dairy cows. Progesterone concentrations also affected preovulatory follicle diameter, making it difficult to measure effects on ovulatory response. Increasing the dose of GnRH did not increase LH release, whereas pretreatment with estradiol did; unfortunately, neither increased ovulatory response. Results have important implications for the use of GnRH-based TAI protocols in heifers. At present, the only alternative is to add a progesterone-releasing intravaginal insert between the first injection of GnRH or pLH and the injection of PGF in order to achieve acceptable pregnancy rates to TAI in heifers.

Background

Estrus synchronization and artificial insemination (AI) have not been widely used by cow-calf producers due to substantial inputs, including cost of consumables, the time and labor needed for treatments and estrus detection, and unpredictable results. However, AI has been critical to rapid genetic improvement in the dairy industry and could be of great benefit to the beef industry. Timed-AI (TAI; by appointment, without estrus detection) reduces labor costs by eliminating estrus detection, but has resulted in unpredictable pregnancy rates. However, we have already developed functional protocols and have shown that TAI is feasible in beef cattle. The overall objective of this proposal was to facilitate the use of AI in order to utilize genetically superior sires within the beef herd. We proposed to refine protocols, developed largely by ourselves, that synchronize ovulation, resulting in high fertility to TAI, without the need for estrus detection. A series of experiments were conducted to simplify protocols, improve pregnancy rates, and to utilize readily available pharmaceuticals. It was anticipated that the information generated in the proposed studies would improve pregnancy rates following TAI by increasing the proportion of cattle responding to follicle synchronizing treatments and promoting growth and maturation of the preovulatory follicle.

Specifically, we proposed to confirm results suggesting that regimens for synchronization of follicle waves and ovulation could be incorporated into prostaglandin- (PGF) or progestin-based synchronization programs, and that cattle returning to estrus after TAI could be resynchronized to reduce the need for estrus detection associated with reinsemination. In addition, new approaches that might improve synchrony in TAI protocols, e.g. "presynchronization", and alternative pharmaceutical products for the synchronization of follicular wave development and ovulation were investigated. As cost, efficacy and user-friendliness will undoubtedly influence use of AI as a genetic tool, we felt that TAI with high fertility would increase the use of this valuable tool by cattle producers, especially those in the beef industry.

We pioneered the use of CIDR inserts and melengestrol acetate (MGA) in TAI protocols in Canada and the USA. Although we were the first to report that estradiol effectively synchronized follicle development and ovulation for TAI (resulting in high pregnancy rates), most estradiol currently available is compounded through veterinary prescription; it is anticipated that regulatory authorities may force an end to this practice, leaving veterinarians and producers without access to this highly efficacious compound. Therefore, recent studies focused on the use of gonadotropin releasing hormone (GnRH) and porcine luteinizing hormone (pLH) to synchronize follicle development and ovulation; both are available commercially. However, GnRH has proven to be unreliable in heifers and young, postpartum cows, primarily because it does not consistently induce ovulation, which is necessary to synchronize follicle development. We have shown that this can be largely overcome in heifers by the use of exogenous progestins (CIDR and MGA); these nearly double pregnancy rates to TAI. That heifers respond poorly to GnRH remains puzzling and requires further study.

The following report outlines studies conducted since 2004. Progress reports outlined studies conducted over each 12-mo interval; this report will detail studies based on subject area rather than the chronological order in which expeiments were conducted. However, next to each experiment a date (year) will be placed in brackets so that the reviewer will be able to determine when the experiment was conducted (and reported); consequently, it will be readily apparent which experiments were conducted during the last 12 mo.

THE EFFECTS OF ESTRADIOL ON FOLLICULAR WAVE DYNAMICS

Effects of dose of estradiol and progesterone in PGF-treated beef heifers (2004).

Objective - To determine the effects of dose of estradiol benzoate (EB) and/or progesterone on follicular and luteal dynamics in a two-dose, prostaglandin (PGF)-based protocol in beef heifers.

Rationale - Estradiol and progesterone synchronized follicular wave emergence and ovulation in a two-dose, PGF-based synchronization program (Martínez et al., 2004; Theriogenology 62:363-372). However, some heifers displayed estrus prior to the second PGF, suggesting that premature luteolysis occurred. This experiment was designed to investigate whether lower doses of either estradiol or progesterone would result in normal corpus luteum (CL) function.

Methods - In two replicates, beef heifers (n=28) received PGF on Day -7, and were randomly allocated to nine treatment groups on Day 0 to receive 0, 1, or 2 mg of EB and 0, 50, or 100 mg of progesterone in a 3 x 3 factorial design. A second PGF treatment was given on Day +7. Ultrasonography was done once daily from Days -5 to +9, and every 12 h thereafter until ovulation. Blood samples were collected at 12-h intervals from Days 0 to 5 for estradiol and FSH concentrations, and every 24 h for progesterone concentrations.

Results - The day of the follicular wave at the time of treatment tended to vary among groups ($P=0.08$), and the diameter of the dominant follicle also differed ($P<0.05$). There tended to be an effect of EB treatment on the interval from that treatment to follicular wave emergence ($P<0.1$), but there was no effect of progesterone treatment and no interaction (both $P>0.90$). The interval from EB treatment to wave emergence was shorter in heifers that received 2 mg EB (4.6 ± 0.3 d) than in those than received no EB (5.9 ± 0.6 d), whereas the 1 mg EB group (5.1 ± 0.6 d) was intermediate. The diameter of the CL at the time of the second PGF was not affected by EB treatment ($P=0.72$), but was affected by progesterone treatment ($P=0.01$), with a tendency for an interaction ($P<0.06$). The variability of the interval from the second PGF to ovulation tended to be influenced by treatment group ($P<0.1$). There was an effect of time ($P<0.01$) and a tendency for an interaction ($P=0.06$) on plasma progesterone concentrations, whereas plasma estradiol concentrations were affected by EB dose, time and an interaction (all $P<0.01$). Plasma concentrations of FSH were affected by time ($P<0.01$) and tended to be affected by an interaction ($P=0.11$).

In summary, EB treatment 7 d after a single injection of PGF effectively synchronized follicular development, whereas concurrent progesterone treatment appeared to suppress CL function. Therefore, premature estrus in previous studies may have been more a result of progesterone treatment than estradiol treatment. Results did not provide convincing support for the use of estradiol and progesterone in a PGF-based estrus synchronization protocol for TAI. Subsequent experiments with estradiol will include the use of an exogenous progestin (e.g. CIDR or MGA) to account for the possibility of premature luteal regression.

Evaluation of "Estrus®" (estradiol cypionate) in a synchronization program for timed-AI in dairy heifers (2005).

Objective - To evaluate the use of a commercial formulation of estradiol cypionate (Estrus®) to synchronize follicle wave emergence and ovulation in a controlled breeding plan involving TAI in Holstein heifers.

Rationale - ECP (estradiol cypionate), the only estradiol ester available for veterinary use, was recently withdrawn from the Canadian pharmaceutical market. Estrus® is a preparation of estradiol cypionate (2 mg/mL) that a small pharmaceutical company introduced to the Canadian market under the auspices of a drug identification number (DIN). This preliminary study was conducted to determine the efficacy of this product in a TAI protocol.

Methods - Holstein heifers (n = 26), 14-15 mo old and an average body weight of 462 kg and BCS of 5.2 (scale 1 to 9) were used. On Day 0, all heifers (at random stages of the estrous cycle) received a progesterone-releasing vaginal insert and were given 1 mg of estradiol cypionate (Estrus®) to synchronize follicular wave emergence. On Day 9, intravaginal inserts were removed and PGF was given. Heifers received a second injection of Estrus® (0.5 mg) on Day 10 to synchronize ovulation, with TAI 36 h later (60 h after PGF) with commercial frozen-thawed semen from one sire. On Day 0, all animals were examined by transrectal ultrasonography to detect anomalies of the reproductive tract and determine reproductive status. Pregnancy diagnosis was performed by ultrasonography approximately 30 d after AI or rebreeding.

Results - Pregnancy rate to TAI was 46.2% (12/26). Fourteen heifers returned to estrus following AI. Overall, the proportion of nonpregnant heifers returning to estrus between 17 and 24 d after TAI was 92.8% (13/14). Pregnancy rate for return services was 78.6% (11/14).

In summary, pregnancy rates to the TAI were lower than was expected, but pregnancy rates to rebreeding 17 to 24 d after TAI were highly acceptable. In another experiment at the same site involving other pharmaceutical products and TAI, pregnancy rate in beef heifers was even lower (40/150, 26.6%). The reason(s) for the somewhat disappointing pregnancy rates is unknown, but was not considered to be due to the pharmaceutical products used to synchronize estrus and ovulation. We believe that there were nutritional problems that affected pregnancy rates; five different treatment protocols were used and all had low pregnancy rates. Therefore, Estrus® worked as well as any of the other protocols. However, the injectable volume was very small and could cause dosing errors; that a concentration of 0.5 mg estradiol cypionate/mL would be preferable has been communicated to the manufacturer.

FIXED-TIME AI WITH MGA-BASED PROTOCOLS

A comparison of ECP and GnRH in an MGA-based protocol for timed-AI (2004).

Objective - To compare pregnancy rate to TAI in beef heifers treated with ECP or GnRH to synchronize follicle wave emergence and ovulation in an MGA-based protocol.

Rationale – We reported that ECP and GnRH were equally efficacious in synchronizing follicle wave emergence and ovulation for TAI in CIDR-treated heifers (Colazo, et al., 2004;

Theriogenology 61:1115-1124). Furthermore, MGA is an inexpensive means of controlling the estrous cycle. However, in some of our previous studies, pregnancy rates to TAI were more consistent with a CIDR- versus MGA-based protocol (Colazo et al., 2006; Theriogenology 65:557-572).

Methods - Angus and Angus-cross heifers (n=992) were fed MGA (0.5 mg/head/day) for 8 d, and given PGF 24 h after the last feeding of MGA. Half of the heifers received 1 mg ECP at the start of MGA feeding and 0.5 mg ECP 24 h after PGF, with TAI 48 h after ECP. The other half received 100 μg GnRH 48 h after the first feeding of MGA and 100 μg GnRH at TAI (56-58 h after PGF). Pregnancy diagnosis was performed by ultrasonography 30 d after TAI.

Results – Although TAI was initially planned for approximately 32 h after ECP, only a few heifers were in standing estrus at that time in the first replicate (n=110); since many more were in estrus following TAI, heifers in the first replicate were reinseminated 48 h after ECP. The remaining heifers were inseminated once (48 h after ECP). Pregnancy rates were 46.4% (51/110) in the ECP-2AI group, 32.7% (117/358) in the ECP-1AI Group, and 40.3% (211/524) in the GnRH-1AI group. The ECP-treated heifers that were inseminated twice and heifers given GnRH had higher pregnancy rates ($P<0.01$) than those treated with ECP and inseminated only once.

In summary, it was clear that timing of TAI after ECP treatment was not optimum in MGA-treated heifers; previous studies with ECP and CIDR inserts did not reveal this discrepancy. Although combinations of GnRH (Ovsynch/Cosynch protocols) and MGA could offer reasonable alternatives for TAI in beef heifers, the clearance of MGA appeared to affect timing of ovulation.

TIMED-AI IN CIDR-BASED PROTOCOLS

Effect of length of CIDR treatment on pregnancy rate to timed-AI (2004).

Objectives – 1) To compare preovulatory follicle size between 7- and 8-d CIDR protocols; 2) to determine the interval from CIDR removal to ovulation and pregnancy rate for 7- vs 8-d CIDR protocols.

Rationale - All of our previous studies with CIDR for TAI have involved a 7-d protocol. However, Macmillan in Australia and Bo in Argentina claimed an 8-d protocol resulted in higher pregnancy rates (although we have not seen data to support those claims). A 7-d protocol permits all treatments to be done on the same day of the week. However, when follicular wave emergence and ovulation are synchronized with estradiol, an 8-d CIDR protocol would be expected to result in a larger preovulatory follicle and a shorter interval from CIDR removal to estrus and ovulation. Furthermore, a larger preovulatory follicle may increase pregnancy rate.

Methods - Crossbred beef heifers (n=37) and cows (n=42) were randomly assigned to receive either 2 mg estradiol benzoate (EB) and a CIDR on Day 0 (Group 8-d) or Day 1 (Group 7-d). On Day 8, CIDR were removed and PGF was given to both groups. Twenty-four hours later (Day 9), all cattle received 1 mg EB and were time inseminated (TAI) approximately 32 h later (Day 10; 56 h after CIDR removal). Cattle were examined ultrasonically on Day 0 (CIDR insertion and EB treatment), Day 10 (at TAI), and Day 11 (24 h after TAI) to determine preovulatory follicle diameter and the time of ovulation. Ovulation was confirmed by the disappearance of a large (≥10 mm) follicle

detected at the previous examination. Pregnancy diagnosis was performed by ultrasonography 28 d after TAI.

Results - All cattle were cycling at beginning of experiment. Eight cows (19.0%) lost their CIDR (3 and 5 cows from Groups 8-d and 7-d, respectively). Four of those cows ovulated before TAI and were excluded from the statistical analyses. Four heifers ovulated before TAI (two in each group) and were similarly excluded. There was no evidence that an 8-d protocol increased pregnancy rate; in fact, pregnancy rates were numerically higher in heifers (12/19, 63.2% vs 11/18, 61.1%) and statistically higher in cows (17/21, 80.9% vs 9/17, 52.9%; $P<0.05$) with the 7- vs the 8-d protocol, respectively. There was no relationship between ovulatory follicle size (beyond 10 mm) and pregnancy rates in heifers, but there tended to be an effect of follicle size on pregnancy rate in cows ($P<0.09$).

This experiment was followed by a confirmatory experiment involving 267 heifers.

Methods - Crossbred beef heifers at random stages of the estrous cycle received 2 mg EB and a CIDR on Day 0 (n=132; Group 8-d), or Day 1 (n=135; Group 7-d). On Day 8, CIDR were removed and PGF was given to all heifers. Twenty-four hours later (Day 9), all heifers received 1 mg EB and were inseminated on Day 10 (32 h later; 54 to 58 h after CIDR removal). Pregnancy diagnosis was done by ultrasonography approximately 28 d after AI.

Results – Pregnancy rate was 66.6% (90/135) in the Group 7-d and 56.8% (75/132) in the Group 8-d ($P<0.1$). Therefore, the 7-d protocol tended to increase pregnancy rate, at least with the timing of AI used in this experiment.

In summary, there was no justification to increase the duration of CIDR treatment from 7 to 8 d. It may be possible to improve pregnancy rates by fine-tuning the timing of AI in an 8-d protocol, but it is unlikely that pregnancy rates would be much higher that what we have achieved with 7-d protocols.

THE EFFECTS OF PRESYNCHRONIZATION

Effects of PGF presynchronization in estradiol/CIDR or PGF protocols on pregnancy rates to TAI in suckled beef cattle (2004).

Objective - To determine the effects of presynchronization and CIDR treatment on pregnancy rate in lactating beef cattle subjected to a TAI protocol based on estradiol, progesterone and PGF.

Rationale – We previously reported that presynchronization with two injections of PGF 11 d apart in beef heifers improved fertility in a GnRH-based protocol. It is likely that presynchronization resulted in a growing dominant follicle that would be more likely to ovulate following treatment with GnRH. However, the effects of presynchronization on a CIDR-based protocol involving the use of estradiol and progesterone have not been reported.

Methods - Crossbred suckled beef cows (n = 288), 47 ± 14.8 d post-partum, were allocated to three treatment groups on the basis of parity, body weight and body condition score. All cattle were treated with 2.5 mg estradiol-17β and 100 mg progesterone on Day 0 and PGF and 0.5 mg ECP on

Day 7, with TAI on Day 9.3 (56 h after CIDR removal on Day 7). Experimental treatments were CIDR from Days 0 to 7 (Groups 1 and 3), and PGF on Day -7 (Groups 2 and 3). From Days 11 to 54 (i.e., after TAI), the cows were exposed to fertile bulls and frequently observed for estrus and breeding. Pregnancy was diagnosed by ultrasonography (Day 54) and transrectal palpation (Day 129).

Results - There was no interaction of parity and treatment ($P>0.05$). Pregnancy rates to TAI were 46.9% (Group 1 – CIDR), 31.2% (Group 2 – PGF on Day -7), and 45.3% (Group 3 - CIDR plus PGF on Day -7) and to natural service were 46.9, 64.6 and 45.3%. Group 2 (PGF on Day -7) had the lowest pregnancy rate to TAI ($P<0.05$), but the highest pregnancy rate to natural service ($P<0.05$); most natural service occurred from 17 to 24 d after TAI (85.2, 59.1, and 72.4%; $P>0.05$). Cumulative pregnancy rates were similar ($P>0.05$) among groups (93.8, 95.9 and 90.6%, respectively).

In summary, two injections of PGF (without a CIDR) with estradiol treatment in between to synchronize follicle wave emergence and ovulation did not provide sufficient synchrony to facilitate TAI. Although pregnancy rate to TAI was increased significantly with the addition of a CIDR, it was not further affected by PGF presynchronization; therefore, presynchronization has no significant benefit in an estradiol/CIDR protocol.

The effects of presynchronization with a used-CIDR and low progesterone concentrations prior to GnRH treatment on pregnancy rates in a GnRH/CIDR TAI protocol (2004).

Objective – To investigate the effect of low progesterone concentrations prior to a GnRH treatment on pregnancy rate in a GnRH/CIDR protocol to TAI.

Methods - On Day 0, lactating beef cows (n=34) and heifers (n=37) were allocated by replicate to two groups to receive either PGF and a twice-used CIDR for 5 d (Pretreatment; low progesterone), or no pretreatment (Control; high progesterone). On Day 5, used CIDR were removed and all cattle received a new CIDR and 100 μg GnRH. On Day 12, the new CIDR were removed and PGF was given. A second GnRH treatment was given concurrent with TAI on Day 14 (54-56 h after PGF). Transrectal ultrasonography was done to monitor the CL and follicles and subsequently for pregnancy diagnosis (Days 42 to 49).

Results - Diameter of the dominant follicle on Day 5 was larger and more variable in cows than in heifers (15.5 ± 5.6 vs 11.4 ± 3.5 mm; means, $P<0.001$; variance, $P<0.003$), and tended ($P=0.13$) to be larger in the Pretreatment group (14.3 ± 4.9 mm) than in the Control group (12.6 ± 5.2 mm). More Pretreated (60.0%) than Control (36.1%) cattle ($P<0.005$), and more cows (64.7%) than heifers (32.4%; $P<0.03$) ovulated following the first GnRH injection. At the time of TAI, 5 (13.5%) heifers had already ovulated ($P<0.03$). Diameter of the preovulatory follicle was affected by parity ($P<0.001$), but not pretreatment ($P=0.4$), and tended to be larger in heifers that became pregnant ($P=0.13$). Pregnancy rate was not affected by parity ($P=0.71$), or pretreatment ($P=0.34$); however, pretreatment tended to increase pregnancy rate in heifers (63.2 vs 38.9%; $P=0.19$).

In summary, pretreatment with a twice-used CIDR plus PGF (presynchronization) increased the proportion of cattle that ovulated to the first GnRH treatment, but not preovulatory follicle size or

pregnancy rates in cows; however, fertility tended to be improved in heifers. Clearly, presynchronization should be considered for GnRH-based protocols in heifers.

Effects of presynchronization with a used-CIDR and treatment with eCG in a GnRH-based TAI protocol in lactating beef cows (2004).

Objectives - To determine the effects of pretreatment with a used CIDR on follicle size and ovulation rate to an initial GnRH treatment, and the effects eCG (equine chorionic gonadotropin/ pregnant mare's serum gonadotropin) at the time of PGF on preovulatory follicle size and fertility in cows subjected to a GnRH-based TAI protocol.

Rationale – It was presumed that a used CIDR for 2 wk would result in a persistent follicle that should ovulate following administration of GnRH. We previously determined that presynchronization with PGF improved ovulatory response in GnRH-treated heifers, and it was reported (Cutaia, et al., 2003; Theriogenology 59:216) that treatment with eCG concurrent with removal of a progestin-releasing device improved pregnancy rates in estradiol treated cows; eCG appeared to stimulate growth and maturation of the growing dominant follicle, resulting in higher progesterone concentrations. However, the combined effects of presynchronization, a progestin-releasing device, and eCG have not been investigated in lactating beef cows treated with GnRH.

Methods - Lactating, crossbred beef cows (n=292), 2 to 12 yr of age were randomly allocated to two groups to receive either a previously used CIDR for 15 d or no treatment (Control). At CIDR removal (Day 0), all cows received 100 μg GnRH. On Day 7, all cows received PGF and were further allocated to receive either 400 IU of eCG or no treatment (2 x 2 factorial design; n=73 per group). On Day 9 (54 h after PGF), cows were given 100 μg GnRH, concurrent with TAI. Transrectal ultrasonographic examinations were done on a subset of approximately 40 cows in each group on Days –15, 0, 7, and 9 to assess ovarian structures, and on all cows on Day 37 to confirm pregnancy.

Results - Overall, 53% of cows had a CL on Day -15 (P=0.23). At first GnRH treatment (Day 0), fewer CIDR pretreated cows than Control cows had a CL (15.2 vs 85.0%, P<0.001), whereas mean diameters of the dominant follicle were larger in CIDR pretreated cows (18.2 ± 0.4 vs 13.8 ± 0.4 mm, P<0.001). Moreover, the proportion of cows that ovulated following the first GnRH treatment was higher (P<0.001) in CIDR pretreated (75.0%) than Control (48.7%) cows. Eight (10.0%) cows presynchronized with a CIDR did not ovulate and had a luteinized follicle (31.7 ± 1.9 mm) at the time of PGF injection. Although CIDR pretreated cows had larger (P<0.002) dominant (preovulatory) follicles than Control cows on Day 9 (16.6 ± 0.3 vs 15.2 ± 0.3 mm), presynchronization did not affect fertility (53.4 vs 54.1% pregnant, respectively). However, diameter of the dominant follicle at TAI in cows that became pregnant was smaller in CIDR pre-treated than Control cows (15.3 ± 0.3 vs 16.6 ± 0.3; P<0.005). Treatment with eCG on Day 7 did not affect (P=0.17) the diameter of the dominant follicle at TAI, but tended (P=0.06) to increase pregnancy rate (58.9 vs 48.6%), especially in Control cows (P=0.08). Pregnancy rates in Control cows (32/73, 43.8%), Control cows given eCG (47/73, 64.4%), CIDR pretreated cows (39/73, 53.4%) or CIDR/eCG-treated cows (39/73, 53.4%) did not differ. However, pregnancy rate was affected by parity; 2-yr-old cows had a lower (P<0.04) pregnancy rate than older cows (42.9, 58.7, and 58.2% for 2, 3-4, and >5yr, respectively). Treatment with eCG increased pregnancy rate by 33% (P<0.03) in 2-yr-old Control cows.

In summary, presynchronization with a used CIDR prior to a GnRH-based protocol increased the proportion of cows ovulating in response to the first GnRH treatment. Although CIDR pretreated cows had a larger dominant follicle at TAI, pregnancy rate was not improved. Treatment with eCG tended to increase fertility in Control cows, and significantly increased pregnancy rates in younger cows (that were also in poorer body condition and less likely to be cycling), suggesting that eCG may be useful in GnRH-based protocols in lactating beef cows, particularly if there is a large proportion of young and/or anestrous cows.

Effects of CIDR-based presynchronization and eCG on fertility to a GnRH-based timed-AI protocol in beef cattle (2005).

Objectives - To determine the effects of: 1) presynchronization with PGF and CIDR treatment for 7 d prior to the first GnRH treatment; and 2) treatment with eCG (concurrent with PGF treatment) on fertility in beef cattle synchronized with a modified GnRH-based TAI protocol.

Rationale - Presynchronization with PGF prior to the first GnRH injection increased pregnancy rates to TAI in lactating dairy cows. Although presynchronization of beef heifers with PGF reduced the incidence of premature estrus, pregnancy rate to TAI was not improved. In studies conducted in 2004, a 15-d presynchronization treatment with a used-CIDR improved ovulation rate to the first GnRH treatment in lactating beef cows, but pregnancy rate was not correlated to the ovulatory response to first GnRH treatment. Treatment with eCG tended to increase pregnancy rate in lactating beef cows, especially 2-y-olds, subjected to a modified Cosynch protocol.

Methods (Heifers) – Beef heifers (n=447), 12 to 15 mo old, were used at three locations. On Day 0, half of the heifers received a new CIDR and PGF. On Day 7, the remainder received a once-used CIDR and all received 100 μg GnRH. On Day 14, CIDR were removed, PGF was given to all heifers, and they were further subdivided to receive either 300 IU of eCG or no treatment (2 x 2 factorial design). On Day 16 (54-56 h after PGF and CIDR removal), heifers were given 100 μg of GnRH concurrent with TAI. Transrectal ultrasonography was done on Day 0 to assess ovarian structures, and on approximately Day 50 (range, Days 44-69) to confirm pregnancy.

Results (Heifers) - Overall, 72.7% of heifers were puberal on Day 0 (150/150, 5/37 and 170/260 for locations A, B, and C, respectively; $P<0.001$). Pregnancy rate was affected ($P<0.001$) by location (27.3, 62.2. and 51.1% for the three locations), but was not affected ($P=0.3$) by puberal status at Locations B and C. Pregnancy rate was not affected ($P=0.5$) by presynchronization, but tended ($P=0.1$) to be reduced in eCG treatment groups (40.4 vs 47.7%; Figure 1).

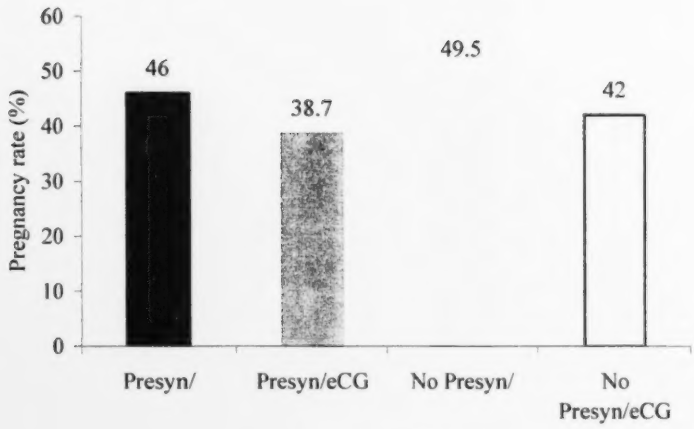

Figure 1. Proportion of heifers (n=447) pregnant by treatment group in a GnRH-based protocol for TAI. Pregnancy rate to TAI did not differ (P=0.5) between presynchronized (Presyn) and non-presynchronized (No Presyn) heifers. Treatment with eCG (on the day of PGF treatment in the Cosynch protocol tended (P<0.1) to be associated with a reduced pregnancy rate.

Methods (Cows) - Lactating, crossbred beef cows (n=411), 2 to 12 yr of age were randomly allocated to receive either PGF and a previously used CIDR for 7 d or no treatment (Control; Day 0). At CIDR removal on Day 7, all cows received 100 μg of GnRH. On Day 14, all cows received PGF and were allocated to receive either 400 IU of eCG or no treatment (2 x 2 factorial design). On Day 16 (54-56 h after PGF), cows were given 100 μg GnRH, concurrent with TAI. Transrectal ultrasonography was done in approximately 65 cows in each group on Days 0, 7, and 14 to assess ovarian structures, and in all cows on Day 42 to confirm pregnancy.

Results (Cows) - Overall, 88.8% of cows had a CL on Day 0. Presynchronization increased both the proportion of cows that ovulated to GnRH treatment on Day 7 (76.7 vs 55.0%; P<0.001) and pregnancy rate (58.2 vs 45.4%; P=0.03). However, eCG treatment did not affect pregnancy rate (P=0.3; Figure 2).

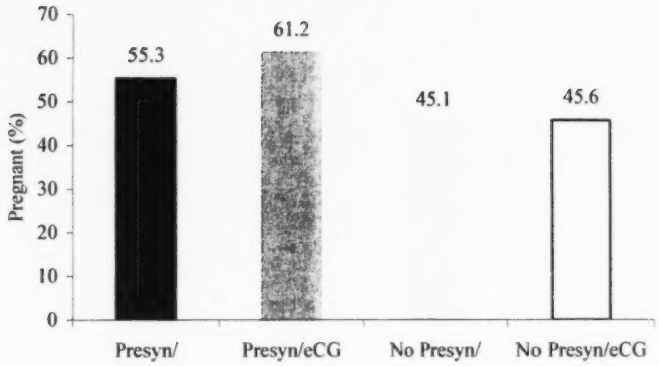

Figure 2. Proportion of cows (n=411) pregnant by treatment group in a GnRH-based protocol for TAI. Pregnancy rate was higher (P=0.03) in cows receiving a presynchronization (Presyn) treatment with a used-CIDR, but treatment with 400 IU of eCG on the day of PGF treatment did not affect (P=0.3) pregnancy rates.

In summary, presynchronization with PGF and a used CIDR (to induce a persistent follicle) prior to a GnRH-based protocol increased ovulation rate to the first GnRH treatment and pregnancy rate in cows but not heifers. In a GnRH-based TAI protocol, eCG did not affect pregnancy rate in cows but tended to reduce pregnancy rate in heifers.

Presynchronization with MGA in a GnRH-CIDR-eCG TAI protocol (2004).

Objective - To evaluate the use of eCG in a GnRH-CIDR protocol in beef heifers that had been presynchronized with MGA.

Methods - Beef heifers (n=127) were fed 0.5 mg/head/day of MGA for 15 d; 12 d after the last feeding (designated as Day 0), heifers received a CIDR and 100 μg GnRH. On Day 7, CIDR were removed, heifers received PGF, and were randomly placed in two groups to receive 300 IU of eCG or no treatment (Control). On Day 9 (54-56 h after PGF), all heifers received 100 μg GnRH, concurrent with TAI. Ultrasonographic examinations were done to monitor the CL and follicles and pregnancy (Days 42 to 49).

Results - Overall, 79.5% of the heifers had a CL, and 9.4% had a luteinized follicle on Day 0. Seventy-eight heifers (61.4%) ovulated following the first GnRH injection, and those that ovulated had less variable preovulatory follicle size (just prior to TAI) than those that did not (13.7 ± 1.7 vs 13.8 ± 2.3 mm; means, P=0.76; variance, P<0.01). However, there was no difference in preovulatory follicle size (P=0.63), or pregnancy rate (49.2 vs 53.1%; P=0.7) for eCG-treated vs Control heifers.

In summary, MGA appeared to be suitable for presynchronization in beef heifers, but treatment with eCG did not increase preovulatory follicle size or fertility in heifers subjected to an Cosynch-CIDR protocol. Evidence is accumulating that eCG is not only not needed in heifers, but may in fact be contraindicated.

Effects of presynchronization with PGF in heifers receiving a Cue-Mate and either pLH or GnRH (2006).

Objective – To determine whether the benefits of presynchronization with PGF and progestin-releasing device treatment are additive in improving pregnancy rates in GnRH- or pLH-based TAI protocols in beef heifers.

Rationale - Although we had shown that presynchronization with PGF increased the numbers of heifers ovulating to GnRH treatment, and the inclusion of a CIDR in the protocol increased pregnancy rates, it was not clear whether the two treatments were additive in increasing pregnancy rates (to TAI) following treatment with either GnRH or pLH.

Methods - Angus heifers (n = 462) in a single drylot were used. Approximately half of the heifers (Presynch; n=226) received two injections of PGF 14 d apart, and synchronization treatments were initiated 11 d after the second injection of PGF, whereas the remainder (Control; n=236) were treated at random stages of the estrous cycle. On Day 0, heifers received a Cue-Mate intravaginal progesterone device and were treated with 100 μg GnRH; n=233) or 12.5 mg pLH; n=229). On Day 7, Cue-Mate were removed and heifers were given PGF and on Day 9 (54-56 h after PGF), GnRH or pLH (same as first treatment) was given concurrent with TAI (frozen-thawed semen of one of three sires). Ultrasonographic examinations were performed in a subset of 182 heifers on Days 0 and 7 for CL and follicle development, and in all heifers on Days 41 to 49 for confirmation of pregnancy.

Results - The proportion of cycling heifers on Day 0 was 90.7% (165/182). Pregnancy rate tended to differ among bulls (46.7, 56.9 and 61.4% for Bulls A, B and C, respectively; $P<0.1$). Heifers that ovulated in response to the first GnRH or pLH injection had a higher pregnancy rate than those that did not (66.3 vs 51.9%, $P<0.03$). In addition, heifers treated with GnRH tended to have higher ovulatory response to first treatment and pregnancy rate to TAI than those treated with pLH (60.9 and 60.5% vs 50.0 and 52.4%, respectively; $P<0.09$). Although ovulatory response to first GnRH or pLH treatment were 46.5 and 63.5% for Control and Presynch groups, respectively ($P<0.02$), pregnancy rates did not differ (59.8 vs 53.1%; $P>0.2$). However, there was an interaction between presynchronization treatment and ovulatory response to the first injection of GnRH or pLH on pregnancy rates ($P<0.02$); pregnancy rate was higher in Control heifers that ovulated (77.5%) to the first injection of GnRH or pLH than in Control heifers that did not ovulate (52.2%), or Presynch heifers that did (59.0%) or did not (51.4%) ovulate.

In summary, Cue-Mate-treated heifers that ovulated to the first GnRH or pLH treatment had higher pregnancy rates to TAI. Although presynchronization with PGF increased ovulation rate, it did not significantly affect pregnancy rate.

The effects of presynchronization with PGF, the inclusion of a CIDR, and eCG treatment on pregnancy rates in a GnRH-based TAI protocol in lactating beef cows (2006).

Objective – To determine the combined benefits of presynchronization with PGF, a CIDR and eCG (equine chorionic gonadotrophin) treatment on pregnancy rates in a GnRH-based TAI protocol in lactating beef cows.

Methods - Angus, Gelbvieh and Simmental cross-bred cows (n=288) were randomized by breed and postpartum interval into four groups in a 2 x 2 factorial design (89.6% were cycling). All cows

were given PGF on Day -11, and 100 μg GnRH, with or without concomitant insertion of a CIDR on Day 0. On Day 7, CIDR were removed and cows were given PGF, with or without concomitant injection of 400 IU of eCG. On Day 9 (54-56 h after PGF), TAI was done, with concomitant injection of 100 μg GnRH. Pregnancy was confirmed by ultrasonography on Day 38 after TAI.

Results - Ultrasonographic examination of 147 cows on Day 7 revealed that 62.4% had ovulated in response to the first GnRH. Pregnancy rates (ultrasonographic examination) on Day 38 did not differ between cows with and without CIDR (52.9 and 51.4%; P>0.64) or eCG treatment (53.5 and 50.7%; P>0.28), in cycling vs anestrous cows (51.6 vs 56.7%; P>0.76), and in cows that had ovulated (58.1%) or did not ovulate (50.0%) after the first GnRH treatment (P>0.58).

In summary, although presynchronization increased the proportion of cows that ovulated to the first GnRH treatment, there was no significant improvement in pregnancy rate. Similarly, there was no support for the use of a CIDR or eCG in a GnRH-based protocol in lactating beef cows. It would seem prudent only to use eCG when there is a likelihood of benefit, i.e. large numbers of young and/or nutritionally stressed and/or anestrus cows.

STRATEGIES TO IMPROVE FERTILITY IN TAI PROTOCOLS IN BEEF CATTLE.

The effects of reduced progesterone concentrations on pregnancy rates in a CIDR-estradiol-based TAI protocol in beef heifers (2004).

Objectives - To determine the efficacy of 1) a single vs split dose of PGF, and 2) a new vs once-used CIDR, on pregnancy rates following TAI in an estradiol-based protocol in heifers.

Hypotheses: A split dose of PGF and/or a once-used CIDR will result in reduced progesterone concentrations and increased pregnancy rates by allowing the ovulatory follicle to grow under higher concentrations of LH, which should increase the diameter of the ovulatory follicle.

Methods - Heifers (n=360) were divided to receive a new or once-used CIDR and 2 mg EB at random stages of the estrous cycle (Day 0), with CIDR removal and administration of PGF on Day 8. Heifers were given 1 mg EB on Day 9 (24 h after CIDR removal) and TAI 28 to 32 h later (52 to 56 h after CIDR removal). At CIDR insertion, half of the heifers in each CIDR group received a half-dose of PGF, with the administration of a half-dose of PGF at CIDR removal; the other half received a full dose of PGF at CIDR removal. It is noteworthy that all heifers were handled four times (CIDR insertion, CIDR removal, second EB treatment and TAI) and all handlings occurred at the same time for both groups. Pregnancy diagnosis (ultrasonography) was done ~Day 30 after TAI.

Results - Only fifty-six (15.5%) heifers did not have a CL at the beginning of treatment; most heifers were pubertal. Location (P=0.6), PGF treatment (split vs full dose; P=0.2), CIDR treatment (new vs once-used; P=0.7), and ovarian status (cycling vs non-cycling; P=0.1) did not affect pregnancy rate (Figure 4). However, a PGF treatment by ovarian status interaction affected pregnancy rate (P<0.01; Figure 3). The split dose of PGF improved pregnancy rates in non-cycling animals, whereas the full dose of PGF improved pregnancy rates in cycling animals.

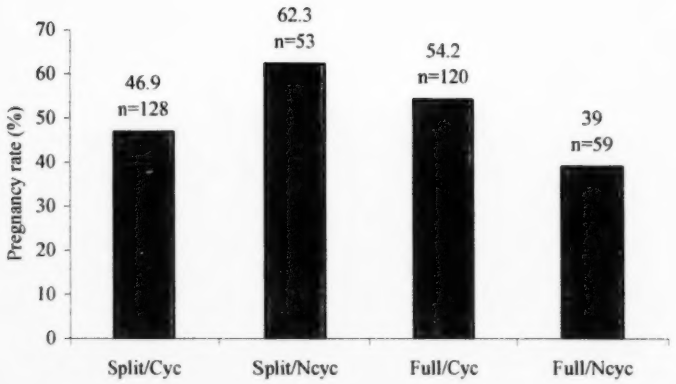

Figure 3. Pregnancy rate by PGF treatment (Split vs Full dose) and ovarian status (Cycling vs Noncycling) at CIDR insertion. All heifers received a new or once-used CIDR and 2 mg EB on Day 0, with CIDR removal and administration of PGF on Day 8. Heifers were treated with 1 mg EB on Day 9 and TAI 28 to 32 h later. At the time of CIDR insertion, half of the heifers in each CIDR group received a half-dose of PGF with the administration of a half-dose of PGF at CIDR removal; the other half received a full dose of PGF at CIDR removal.

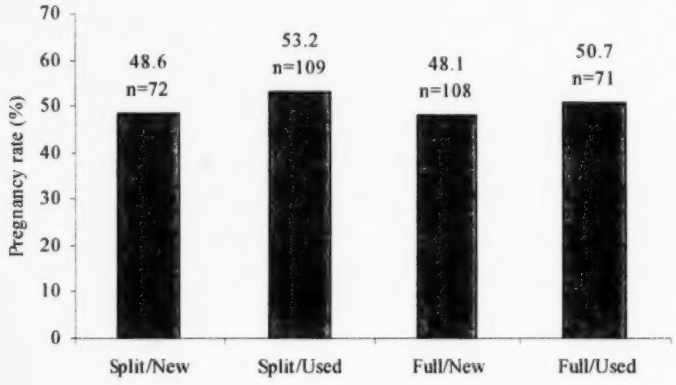

Figure 4. Pregnancy rate by PGF and CIDR treatments. All heifers received a new or once-used CIDR and 2 mg of estadiol benzoate (EB) at random stages of the estrous cycle (Day 0), with CIDR removal and administration of PGF on Day 8. Heifers were treated with 1 mg EB on Day 9 and TAI 28 to 32 h later. At the time of CIDR insertion, half of the heifers in each CIDR group received a half-dose of PGF with the administration of a half-dose of PGF at CIDR removal; the other half received a full dose of PGF at CIDR removal.

In summary, the full dose of PGF was expected to be effective in cycling animals, but the apparent benefit of a split dose of PGF in non-cycling animals was unexpected. It is unknown how a split dose of PGF affected non-cycling heifers, unless the first injection induced cyclicity; although this has been alluded to in other studies, the mechanism remains unclear.

The effect of progestin treatment following TAI on pregnancy rates (2006).

Objective – To determine whether progestin treatment after TAI will increase pregnancy rates to that breeding.

Rationale – There are conflicting reports regarding the use of CIDR to increase circulating progesterone concentrations (and pregnancy rates) in previously inseminated cattle. We have shown the benefit of a used-CIDR in resynchronizing cattle after TAI. We proposed to combine ideas, i.e., increase pregnancy rates by increasing progesterone concentrations and concurrently resynchronize returns to estrus.

Methods - Five days after TAI (n=288), half of the lactating beef cows (blocked by synchronization treatment) received a Cue-Mate intravaginal progesterone releasing device and the remainder served as untreated controls. Twenty days after TAI, Cue-Mate were removed and all animals that were detected in estrus were reinseminated. The effects of treatments on pregnancy rates to the TAI, resynchrony and reinsemination were determined 30 and 60 d after TAI.

Results - The TAI pregnancy rates did not differ among synchronization treatments (52.9%) or with (50.3%) or without (53.8%) Cue-Mate for resynchronization ($P>0.05$). However, rebreedings were more synchronous and over a short interval of time in the Cue-Mate resynchronized animals.

In summary, the addition of CIDR or eCG to a modified Cosynch protocol, and post-TAI progestin treatments did not significantly increase pregnancy rates to TAI. However, synchrony of returns to estrus in lactating beef cattle was improved with post-TAI progestin treatment.

FIXED-TIME EMBRYO TRANSFER

Pregnancy rate following transfer of embryos to LH-treated recipients (2004).

Objective - To evaluate the effect of pLH (Lutropin-V) treatment prior to embryo transfer on pregnancy rates in recipients receiving in vivo- or in vitro-produced bovine embryos.

Methods - Heifers (n = 37) and lactating (n = 28) and non-lactating (n = 150) beef cows (at random stages of the cycle) were given 100 μg GnRH on Day -9, PGF on Day -2 and GnRH on Day 0 (66 h after PGF; without estrus detection). Cattle were randomly allocated, by class, into three groups: no further treatment (Control; n = 71), or 12.5 mg pLH on Day 5 (n = 72) or Day 7 (n = 72) after the second GnRH. On Day 7, cattle with a CL >10 mm in diameter (determined ultrasonically) received in vivo-produced, fresh (Simmental) or frozen (Holstein), or in vitro-produced, frozen (Holstein) embryos (embryo types were balanced among groups). Pregnancy was determined by ultrasonography on Day 35.

Results - Twenty cattle (9.3%) did not receive embryos; five heifers had cervical abnormalities, and five heifers and 10 cows did not have a CL >10 mm. Overall, 7.1% of the recipients had two CL on the day of embryo transfer. There was no effect ($P>0.05$) of treatment, embryo type (or interaction) or class of recipient on pregnancy rate (overall, 44.1%, 86/195). Similarly, mean (± SD) CL diameter and luteal area did not differ ($P>0.05$) among groups or between pregnant and nonpregnant recipients (overall, 22.0 ± 3.4 mm and 352.0 ± 108.7 mm^2, respectively). However, recipients with a

CL diameter ≥18 mm tended (P<0.1) to have a higher pregnancy rate (45.8 vs 25.0%). In a subset of 40 recipients examined ultrasonically on Day 12, 50% of those treated with pLH on Day 5 and 70% of those treated on Day 7 had two CL, suggesting a potential increase in circulating progesterone concentrations.

In summary, overall pregnancy rate in GnRH-synchronized recipients receiving in vitro- or in vivo-produced embryos by nonsurgical transfer was 44.1%. Embryo survival to Day 35 was not affected by type of embryo or treatment with pLH 5 or 7 d after ovulation. Results did not support previous reports that giving hCG on Day 5 after TAI would increase pregnancy rates.

Effects of a CL from a persistent follicle on fertility of embryo transfer recipients (2005).

Objective - To compare CL diameter, progesterone concentration, and fertility in recipients induced to ovulate a persistent versus a viable dominant follicle.

Hypotheses - Recipients with persistent follicles before GnRH treatment will have larger CL, higher progesterone concentrations and greater fertility following embryo transfer.

Methods - On Day 0, cows (n=120) were given a once-used CIDR and an injection of PGF. Half of cows (n=60) received 100 μg GnRH on Day 7. The cows given GnRH were considered the Control group, whereas the others were expected to develop a persistent follicle (large follicle that forms in cattle with low progesterone concentrations for several days). On Day 14, the CIDR were removed and PGF was given to all cows. Three days later (Day 17), 100 μg of GnRH were given to induce ovulation; this was considered the day of estrus. On Day 24, an in-vitro-produced, frozen-thawed embryo was transferred to all recipients with a functional CL. Cows were examined by transrectal ultrasonography for CL and follicle development (Days 0, 14, and 24), and confirmation of pregnancy (Day 52).

Results - Recipients in the persistent follicle group had a larger ovulatory follicle at the time of PGF treatment and CIDR removal (P<0.001), but CL size, numbers of recipients selected for embryo transfer, and pregnancy rate did not differ between groups (Tables 1 and 2).

Table 1. Ovarian structures and proportion of cows selected as recipients following induction of ovulation of a normal (Control) or persistent preovulatory follicle with GnRH.

	Control	Persistent follicle
No. cows	60	60
No. cows with a CL		
on Day 0 (CIDR insertion; %)	34 (56.6)	36 (60.0)
on Day 14 (CIDR removal; %)	50 (83.3)[a]	13 (21.6)[b]
Dominant follicle size (mm)		
on Day 14 (CIDR removal)	15.5 ± 6.1[ax]	18.8 ± 4.6[by]
No. ≥ 10 mm on Day 14 (%)	11 (18.3)[a]	47 (78.3)[b]
No. suitable recipients (%)	58 (96.6)	56 (93.3)

[ab] Within a row, means and proportions differed (P<0.001)
[xy] Within a row, variances differed (P=0.02)

Table 2. CL diameter, luteal tissue area and pregnancy rate in recipients following induction of ovulation of a normal (Control) or persistent preovulatory follicle with GnRH.

	Control	Persistent follicle
No. cows	58	56
CL diameter (mm)		
on Day 24 (embryo transfer)	24.8 ± 4.6^{x}	23.6 ± 5.7^{y}
Range (mm)	17 – 35	15 - 44
Luteal tissue area (mm^2)	$404 \pm 123^{a}.0$	365.4 ± 130.6^{b}
No. cows pregnant		
on Day 52 (%)	12 (20.7)	13 (23.2)

[ab] Within a row, means tended to differ (P=0.1)
[xy] Within a row, variances tended to differ (P=0.07)

However, recipients that were cycling at the time of initiating synchronization treatments had a higher pregnancy rate (P<0.04) than those that were not cycling (Figure 5).

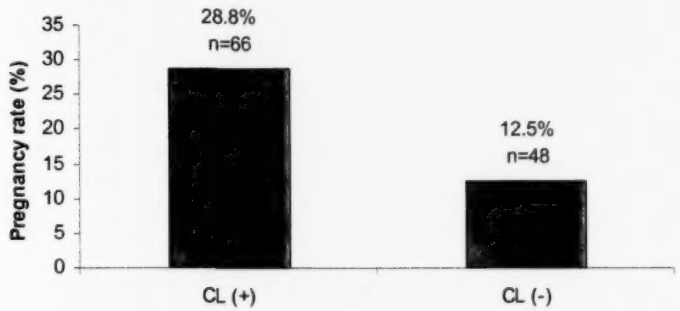

Figure 5. Pregnancy rate in recipients according to cyclicity (CL+ vs CL-) on Day 0 (beginning of experiment) following induction of ovulation of a normal (Control) or persistent preovulatory follicle with GnRH (difference between groups, P<0.04).

In summary, CL that formed from a persistent follicle were no more functional than that from a normal preovulatory follicle in supporting pregnancy in embryo transfer recipients, nor was it any less functional; pregnancy rates did not differ. However, recipients that were induced to cycle by the treatment protocol were less able to maintain pregnancy after embryo transfer.

The use of estradiol-CIDR and eCG to synchronize recipients for transfer of frozen-thawed bovine IVF embryos (2005).

Objective: To investigate the use of eCG in a fixed-time embryo transfer protocol for recipients receiving in-vitro-produced (IVP) bovine embryos.

Methods - In the first experiment, 372 cross-bred recipients were observed for estrus, occurring spontaneously (Group 1) or following a single injection of PGF, with nonsurgical embryo transfer

7.5 or 8.0 d later. Cows in Groups 3, 4 and 5 were treated with a CIDR plus 2 mg EB on Day 0, PGF on Day 5, CIDR removal on Day 7 and 1 mg EB 24 h later (Day 8), with nonsurgical transfer of IVP embryos 8.5 d later (Day 16.5), without estrus detection. Cows in Group 3 received no further treatment, whereas cows in Group 4 received 400 IU eCG on Day 5, and cows in Group 5 received eCG on Day 7. Pregnancy diagnosis (ultrasonography) was done 30 d later.

Results - Although pregnancy rates, based on numbers of recipients synchronized, numerically favored the administration of eCG on Day 5, there were no differences (P=0.40) among groups (Group 1 – 15.4%, n=52; Group 2 – 20.0%, n=50; Group 3 – 19.2%, n=99; Group 4 – 28.1%, n=96; Group 5 – 21.3%, n=75).

Methods - In a second experiment, cross-bred recipients were synchronized with a CIDR plus EB on Day 0, PGF on Day 5, CIDR removal on Day 7, and a second injection of EB on Day 8, and were randomly assigned to receive no further treatment (Group 1; n=400) or an injection of 400 IU eCG on Day 5 (Group 2; n=391). Recipients with a functional CL received a frozen-thawed IVP embryo by nonsurgical transfer on Day 16.5 (8.5 d after the second injection of estradiol) without estrus detection, as in the first experiment. Pregnancy diagnosis (ultrasonography) was done 30 d later.

Results – Pregnancy rates were not affected by CL size, cow age, or embryo quality (P=0.30). Based on recipients that received embryos, pregnancy rates did not differ (P=0.5) between groups (Group 1 – 21.4%, n=154; Group 2 – 24.5%, n=290), but overall pregnancy rates (based on the total number of recipients synchronized) were higher (P<0.001) in Group 2 (eCG; 18.2%) than in Group 1 (no eCG; 8.3%), due to a higher (P<0.03) percentage of recipients used following treatment with eCG (74.2% vs 38.5%).

In summary, the estradiol-CIDR-estradiol protocol facilitated fixed-time embryo transfer in recipients without the need for estrus detection and without compromising pregnancy rates. The administration of eCG on Day 5 of an 8-d synchronization protocol for fixed-time embryo transfer of frozen-thawed bovine IVP embryos improved pregnancy rates in cross-bred beef recipients, especially those that were nutritionally stressed. It would seem prudent to investigate whether the same benefit of eCG occurs when recipients (for fixed-time embryo transfer) are synchronized with GnRH plus PGF, with or without a CIDR.

The effects of CIDR and eCG treatment in a GnRH-based synchronization scheme for fixed-time embryo transfer (2006).

Objective – To determine the effects of eCG and a CIDR on pregnancy rates in a GnRH-based protocol in recipients for fixed-time embryo transfer.

Rationale – We showed that eCG increased pregnancy rates in recipients synchronized with a CIDR and estradiol for fixed-time embryo transfer by increasing the number of recipients considered suitable to receive an embryo. However, estradiol is only available by prescription; there are no data available regarding the use of eCG in GnRH-based protocols.

Methods - A total of 151 lactating cross-bred cows were given PGF on Day -12, 100 ug GnRH on Day 0, with or without concomitant insertion of a CIDR. On Day 3 (expected day of follicle wave emergence), half the cows were given 400 IU eCG. On Day 7, CIDR were removed and cows were given PGF; on Day 9 (54-56 h after PGF), all cows were given 100 μg GnRH. On Day 15,

ultrasonography was done to select suitable recipients for transfer of frozen-thawed IVP embryos on Day 16.

Results - Recipient selection rates were not different between cows given or not given a CIDR (93.4% vs 85.5%; P>0.27) or eCG (91.0 vs 87.8%; P>0.67). In addition, pregnancy rates on Day 43 did not differ whether cows received or did not receive a CIDR (32.3 vs 32.4%; P>0.52) or eCG (35.2 and 29.2%; P>0.21).

In summary, the addition of a CIDR or eCG to a GnRH-based synchronization protocol initiated after PGF presynchronization in lactating beef recipients yielded no improvement in recipient selection rates, or pregnancy rate following fixed-time embryo transfer. As with TAI, the lack of benefit to the use of eCG may be related to the types of recipients used (e.g., young, nutritionally stressed or not cycling), or inappropriate timing of treatment.

THE EFFECTS OF GNRH ON FOLLICLE WAVE DYNAMICS

Effects of progesterone on ovulatory response to GnRH in beef heifers (2004).

Objective - To determine the effects of blood progesterone concentrations on ovulatory response to GnRH treatment in virgin heifers.

Rationale – GnRH-based protocols have been more efficacious in cows than in heifers. Ovulation occurred in 85% of lactating dairy cows treated with GnRH, and approximately 54% of heifers (Pursley et al., 1995; Theriogenology 44:915-923). However, follicular wave emergence will not be synchronized if ovulation is not induced. In a previous study, we showed that as the CL developed, GnRH was relatively less efficacious in inducing ovulation in beef heifers (Martinez et al., 1999; Animal Reproduction Science 57:23-33). We hypothesized that the differences between cows and heifers may due to differences in blood progesterone concentrations, and/or sensitivity of the heifer's hypothalamus to the suppressive effects of progesterone. We predicted that heifers with high progesterone concentrations at the time of the first GnRH injection would be less likely to ovulate (in response to the GnRH) than heifers with average or low progesterone concentrations.

Methods - Forty pubertal heifers received PGF to induce estrus; only heifers detected in estrus were used. Four days after estrus (designated Day 0, ~3 d after ovulation), heifers were randomly allocated to one of three treatment groups to receive no treatment (Control, n=10), a once-used CIDR (Low-P4, n=10), or two once-used CIDRs (High-P4, n=10). Heifers in the Low-P4 group were given two injections of PGF (AM and PM) on Day 1 to induce regression of CL. On Day 2, all heifers received 100 μg GnRH. At the time of GnRH treatment, expected blood progesterone concentrations were: physiological in Control heifers (Day 6 of the estrous cycle); sub-physiological heifers in the Low-P4 group; and supra-physiological in the High-P4 group. Seven days later (Day 9), all heifers received PGF, and CIDR were removed from the Low-P4 and High-P4 groups. Forty-eight hours later (Day 11), all heifers received a second injection of GnRH. Ultrasonographic examinations were performed daily in all heifers (n=30) from Day 0 (day of CIDR insertion) until 2 d after CIDR removal (to monitor ovarian follicles and the CL), and thereafter, every 12 h (in a subset of 19 heifers) to determine the time of ovulation.

Results - The incidence of ovulation in response to the first injection of GnRH is shown in Table 3. Although there was no difference in the ovulation rate between heifers in the Control versus the

Low-P4 group, ovulation rates in both of these groups were higher (P<0.01) than in the High-P4 group. In addition, the interval from treatment to follicular wave emergence was longer (P<0.01) in the High-P4 group than in the other two groups. There was a tendency (P=0.08) for the mean interval from the second GnRH injection to ovulation to be different between the Low-P4 group and the other two groups. Furthermore, there was considerable variation in the time of ovulation in the Low-P4 group, versus no variation in the other two groups.

Table 3. Diameter of dominant follicle on Days 0 and 2 (DF_0 and DF_2, respectively), diameter of the CL on Day 0 (CL_0), incidence of ovulation, and follicular dynamics in GnRH-treated heifers with different plasma progesterone concentrations (continuous variables are mean ± SD).

	Control	Low-P4*	High-P4
No. heifers	10	10	10
Diameter (mm):			
DF_0[1]	9.1 ± 0.7	9.9 ± 1.5	8.4 ± 1.7
CL_0	13.3 ± 2.9	11.8 (± 2.3	12.0 ± 1.5
DF_2	12.1 ± 1.0[a]	11.5 (± 0.7[a]	10.1 ± 0.7[b]
Growth of DF from Days 0 to 2 (mm)[1]	3.0 ± 1.1	1.6 ± 1.3	1.7 ± 1.8
No. heifers ovulating to first GnRH injection (%)	10 (100%)[a]	9 (90%)[a]	3 (30%)[b]
Follicular wave emergence (d)	2.0 ± 0.5[ax]	1.8 ± 0.4[ax]	3.0 ± 1.2[by]
Range (d)	1.0 - 3.0	1.0 - 2.0	2.0 - 5.0
No. heifers ovulating to 2nd GnRH	7	7	5
Interval (h) 2nd GnRH to ovulation[2]	36 ± 0.0	24 ± 17.0	36 ± 0.0

[ab]Means and proportions within a row differed (P<0.01); [xy]variances within a row differed (P<0.01); means within a row tended to differ [1](P=0.07) and [2](P=0.08); * one heifer was excluded from the analysis of day of follicular wave emergence because she did not have a new follicular wave within the observation period.

In summary, although this experiment did not definitively explain why heifers are less responsive than cows to an injection of GnRH, it provided the background for future studies. In heifers with high concentrations of progesterone, it is not clear whether the ovulation rate was lower because of the sensitivity of the heifer's hypothalamus to progesterone, or because the dominant follicle was smaller and less responsive to GnRH-induced LH release.

Effects of plasma progesterone concentrations on LH release and ovulation in beef cattle given GnRH (2005).

Objective - To determine the effects of plasma progesterone concentrations on LH release and ovulation in cows and heifers given GnRH.

Hypothesis - The pituitary gland in virgin heifers is more sensitive to the suppressive effects of progesterone than that of cows; therefore, there will be a greater release of LH and consequently a higher occurrence of ovulation following an injection of GnRH in heifers with low progesterone concentrations.

Methods - Lactating beef cows (n=20) and virgin beef heifers (n=20) were given a CIDR for 7 d and PGF at CIDR removal. Estrus was determined by visual observation and designated as the beginning of the experiment (Day 0). On Days 4 to 6, ultrasound-guided follicular ablation was done in all cattle to synchronize follicular wave emergence. Cattle were then randomly allocated to one of two groups (10 cows and 10 heifers/group) to receive a once-used CIDR with either no further treatment (High-P4), or with two PGF treatments, 12 h apart (Low-P4). All cattle received 100 μg GnRH 6 d after follicular ablation.

Ovarian ultrasonography was done daily from follicular ablation to ovulation or for 4 d after GnRH treatment, to determine ovarian follicular dynamics and time of ovulation. Blood samples (for plasma progesterone concentrations) were taken daily from the day of follicular ablation to ovulation or to 4 d after GnRH treatment; intra-assay coefficients of variation were 3.0, 0.3 and 5.7 % for means of 1.86, 3.02 and 13.53 ng/mL, respectively, and inter-assay coefficients of variation were 5.1, 3.8 and 4.0 % for means of 1.67, 2.91 and 13.20 ng/mL. In a subset of 12 animals (three from each treatment group), blood samples were taken every 30 min for 12 h after GnRH treatment to measure peak LH concentrations. Plasma LH concentrations were determined by radioimmunoassay; intra-assay coefficients of variation were 5.9 and 3.2 % for means of 0.39 and 0.92 ng/mL.

Results - One cow with incomplete luteolysis and two heifers (one had a short luteal phase and the other did not develop a dominant follicle) were excluded from statistical analyses. Plasma progesterone concentrations for the day preceding, the day of, and the day following GnRH treatment did not differ ($P=0.7$) between cows and heifers in the High-P4 groups (6.7 ± 1.0 and 7.0 ± 0.9 ng/mL, respectively) or Low-P4 groups (1.7 ± 0.7 and 2.0 ± 0.7 ng/mL, respectively). However, plasma progesterone concentrations differed ($P<0.01$) between the High-P4 and Low-P4 groups (overall means of 6.8 ± 0.9 ng/mL and 1.9 ± 0.7 ng/mL; Figure 6).

The GnRH-induced LH surge did not differ between cows and heifers ($P=0.3$). However, the GnRH-induced LH peak was lower and of shorter duration in the High-P4 group than in Low-P4 group ($P<0.001$; Figure 6). For LH concentrations, there was an interaction ($P=0.05$) between cows and heifers and High-P4 and Low-P4; cows in the Low-P4 group had a greater ($P=0.02$) area under the LH curve (AUC; 66.7 ng/mL) and a higher peak concentration (27.7 ± 6.5 ng/mL) of LH than cows in the High-P4 group (17.8 and 5.0 ± 1.3 ng/mL), and heifers in either the Low-P4 (43.8 and 18.1 ± 4.1 ng/mL) or the High-P4 groups (26.2 and 10.8 ± 5.8 ng/mL). Thus, peak concentrations of LH and AUC in heifers in the Low-P4 group did not differ ($P=0.2$) from heifers in High-P4 group, but both were greater ($P<0.01$) than in cows in the High-P4 group (Figure 7).

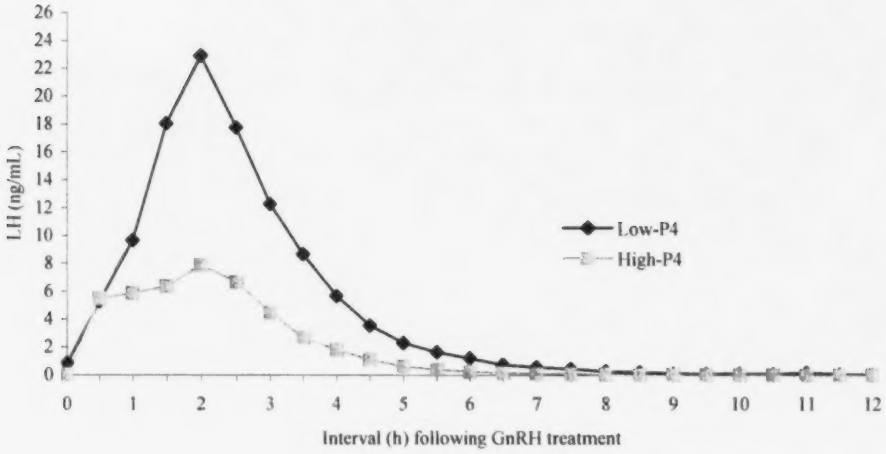

Figure 6. Mean plasma LH concentrations (ng/mL) following an injection of 100 μg GnRH 6 d after follicle ablation in beef cattle (n=12) with high (High-P4) and low (Low-P4) plasma progesterone concentrations. The GnRH-induced LH surge was lower and of shorter duration (P<0.001) in the High-P4 group than in Low-P4 group.

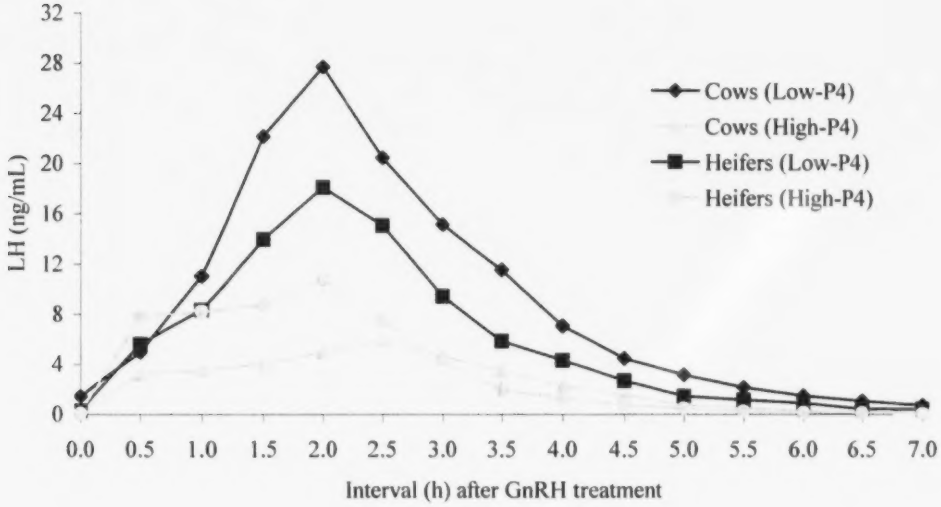

Figure 7. Mean plasma LH concentrations (ng/mL) following an injection of 100 μg GnRH in beef cows (n=6) and heifers (n=6) with high (High-P4) or low (Low-P4) progesterone concentrations. Cows with Low-P4 (♦) had a greater (P<0.02) area under the curve (AUC) and higher (P<0.002) LH peak concentration than animals in the other three treatment groups. The AUC and LH peak concentrations did not differ (P=0.2) between heifers with low (■) or high ( ) plasma progesterone concentrations, but both were higher (P<0.01) than cows with high plasma progesterone concentrations.

On the day of GnRH treatment, the dominant follicle was larger in cows than heifers (11.0 ± 1.1 vs 10.3 ± 0.9 mm; P=0.05), and there was a tendency for the dominant follicle in heifers in the High-P4 group to be smaller than that in heifers in the Low-P4 group (10.3 ± 1.0 vs 11.0 ± 1.0 mm; P=0.06). Ovulatory response to GnRH treatment did not differ (P=0.9) between heifers (14/18, 77.7%) and cows (15/19, 78.9%), but combined for heifers and cows, was lower (P<0.01) in the High-P4 group (11/18, 61.1%) than the Low-P4 group (18/19, 94.7%).

In summary, higher plasma progesterone concentrations reduced LH release and proportions of cattle ovulating in response to GnRH treatment. There was no difference between heifers and cows in LH release or ovulatory response following administration of GnRH; lactating beef cows appeared to be more similar to heifers than lactating dairy cows in ovulatory response to GnRH. Progesterone concentrations affected both preovulatory follicle diameter and the release of LH following administration of GnRH, regardless of the class of animals treated. Results have important implications for the use of GnRH in fixed-time AI protocols in beef cattle.

Effects of dose of GnRH on the induction of ovulation in beef heifers (2006).

Objective – To determine the effect of an increased dose of GnRH on LH release and ovulation in heifers with elevated or low plasma progesterone concentrations.

Rationale - Progesterone clearly affected LH release and ovulation (and thus follicular wave emergence) following the administration of GnRH in previous studies. We hypothesized that higher doses of GnRH would be more efficacious in inducing LH release and ovulation, and in the synchronization of follicle wave emergence for TAI protocols in beef heifers.

Methods – Five days after ovulation, 40 postpubertal heifers underwent follicle ablation and received a once-used CIDR; animals were then randomly assigned to receive an injection of PGF (Low-P4) or no treatment (High-P4) at that time. Follicular wave emergence was expected to occur on Day 7; those in the Low-P4 group were given another injection of PGF at that time to ensure luteal regression. It was expected that the Low-P4 and High-P4 groups would have sub-physiological and high-physiological concentrations of circulating progesterone, respectively, during dominant follicle development. On Day 12, when a 6-d, growing dominant follicle was expected, half the animals in each group received 100 μg GnRH, and the remainder received 200 μg GnRH. Blood samples were taken for progesterone, estradiol and LH concentrations and ultrasonography was done to detect ovulation and follicle wave emergence.

Results - The mean (±SEM) diameter of the dominant follicle at the time of GnRH treatment was smaller in the High-P4 (8.2 ± 0.5 mm) than in the Low-P4 (11.7 ± 0.9) group (P<0.01). There was an effect of progesterone (P=0.012), but no effect of GnRH dose (P=0.60), nor interaction (P=0.77) on the diameter of the dominant follicle at the last scanning prior to ovulation. Ovulation rates were note different among groups; 6/12 (50%) of High-P4 heifers ovulated (whether they were treated with 100 or 200 μg of GnRH). Four of six heifers in the Low-P4 group treated with 100 μg GnRH ovulated, whereas five of six given 200 μg of GnRH ovulated.

Plasma progesterone concentrations did not differ (P=0.14) between Low-P4 (3.2 ± 0.3 ng/mL) and High-P4 (3.0 ± 0.2 ng/mL) groups at the time of CIDR insertion. However, plasma progesterone concentrations at the time of GnRH treatment were higher in the High-P4 (6.0 ± 0.3 ng/mL) than in the Low-P4 (2.4 ± 0.3 ng/mL) group (P<0.01). There was no effect of plasma progesterone, GnRH

dose, nor interactions, on mean plasma LH concentrations after the GnRH treatment (P>0.1), but there was and effect of time (P<0.01; Figure 8).

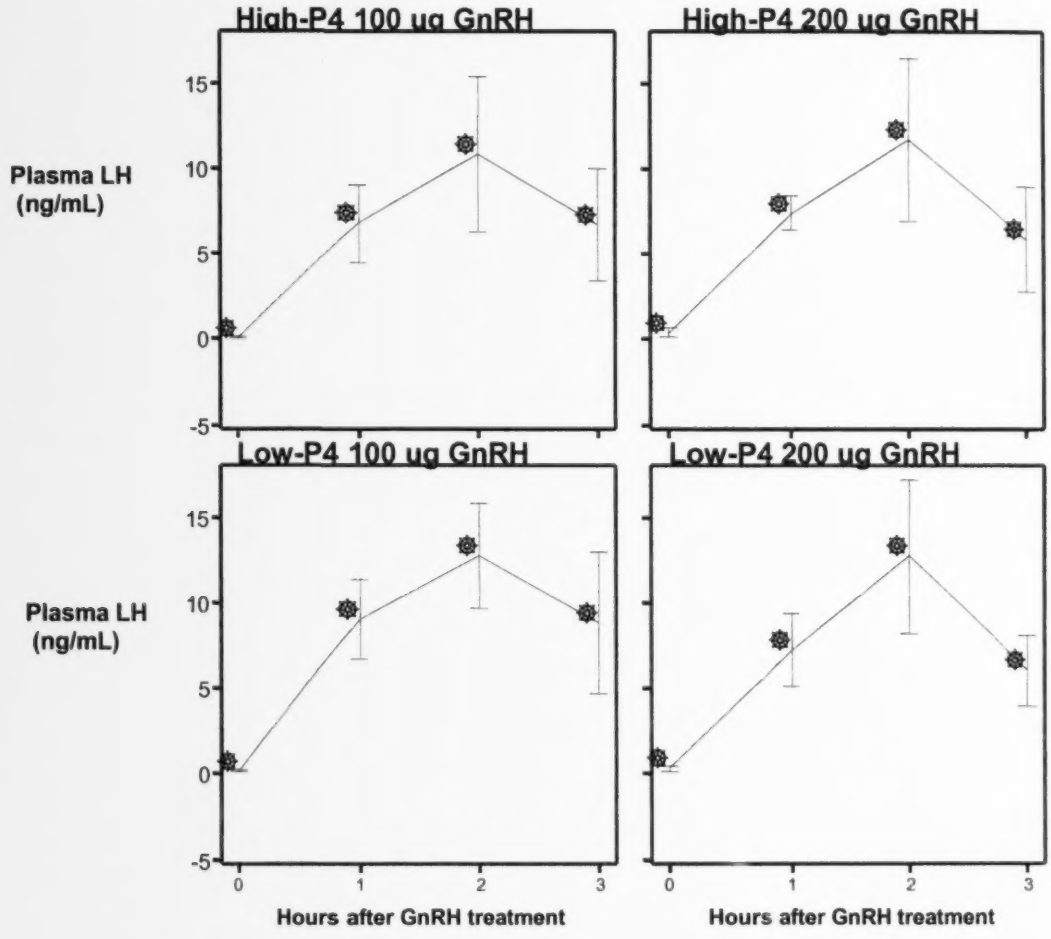

Figure 8. Mean (±SEM) plasma concentrations of luteinizing hormone (LH) in heifers with high (High-P4) or low (Low P4) plasma progesterone concentrations and treated with 100 or 200 μg GnRH. There was no effect of progesterone concentration or dose of GnRH on LH release.

In summary, neither circulating progesterone concentrations nor dose of GnRH affected circulating LH concentrations or ovulatory response. The difference in the effect of circulating progesterone concentration between this experiment and previous experiments is not clear. However, there would appear to be no benefit to increasing the dose of GnRH.

Effects of estradiol on response to GnRH treatment (2006).

Objective – To determine the effect of increased estradiol concentrations on LH release (and ovulation) in response to GnRH treatment in heifers with elevated progesterone concentrations.

Rationale – Estradiol increases the pituitary sensitivity to GnRH by increasing the number of GnRH receptors. Elevated progesterone concentrations suppress LH release and maximum diameter of the dominant follicle (see 2004 and 2005 studies). We hypothesized that the suppressive effects of

progesterone on LH release following administration of GnRH may have been due to suppression of estradiol production in LH-deprived developing follicles. Exogenously administered estradiol may overcome the suppressive effects of progesterone.

Methods – Five days after ovulation (Day 5), 28 postpubertal heifers underwent follicle ablation and received a once-used CIDR and were randomly assigned to receive an injection of PGF (Low-P4) or no treatment (High-P4). Follicle wave emergence was expected to occur on Day 7; those in the Low-P4 group were given another injection of PGF at that time to ensure luteal regression. On Day 11, when a growing dominant follicle was expected, half of the heifers in each group received 0.25 mg estradiol benzoate im and 12 h later (Day 6), all animals received 100 μg GnRH. Blood samples were taken for progesterone, estradiol and LH concentrations, and ultrasonography was done to determine dominant follicle growth and ovulation, and emergence of new wave (after ovulation).

Results - Characteristics of the follicular wave and dominant follicle data are shown in Table 4. There was an effect of progesterone ($P<0.05$; $P<0.01$), but no effect of estradiol ($P=0.21$; $P=0.40$), and no interaction ($P=0.36$; $P=0.37$) on the diameter of the dominant follicle at the time of the GnRH treatment or at the last scanning prior to ovulation, respectively. All heifers in the Low-P4 group (11/11) ovulated in response to treatment with GnRH, whereas only 7/11 heifers in the High-P4 group ovulated (3/6 ovulated in the High-P4-estradiol group, whereas 4/5 ovulated in the High-P4-no estradiol group) suggesting that estradiol may have interfered with ovulation.

Table 4. Characteristics (mean ± SEM) of the follicular wave and dominant follicle in heifers with high (High-P4) or low (Low-P4) progesterone concentrations, with or without estradiol benzoate treatment 12 h prior to GnRH.

End points	High-P4	Low-P4	Probability*
Interval from spontaneous ovulation to day of follicular ablation (d)			
Day after ovulation	6.6 ± 0.4	6.5 ± 0.2	0.81*
Interval from follicular ablation to wave emergence (d)			
Day	1.2 ± 0.2 (1-3)	0.9 ± 0.1 (0-1)	0.2
Diameter of the dominant follicle at			
GnRH treatment (mm)	11.1 ± 0.6	13.6 ± 0.6	0.027
Prior to ovulation (mm)	11.2 ± 0.4	14.0 ± 0.5	0.002

* Mann-Whitney test.

Plasma progesterone concentrations did not differ ($P=0.21$) between Low-P4 (5.2 ± 0.6 ng/mL) and High-P4 (4.3 ± 0.5 ng/mL) groups at the time of CIDR insertion. However, plasma progesterone concentrations at the time of GnRH treatment were higher in the High-P4 (8.7 ± 0.8 ng/mL) than in the Low-P4 (1.8 ± 0.2 ng/mL) group ($P<0.01$). There was an effect of estradiol treatment ($P<0.05$) and time ($P<0.01$), but no effect of progesterone ($P=0.07$), nor interactions ($P=0.15$) on mean plasma LH concentrations after the GnRH treatment (Figure 9). Plasma LH concentrations 1 h after

GnRH treatment were higher (P=0.01) in heifers that received estradiol (22.8 ± 2.0 pg/mL) than those heifers that did not receive estradiol (11.7 ± 3.5) 12 h prior to GnRH (Figure 10).

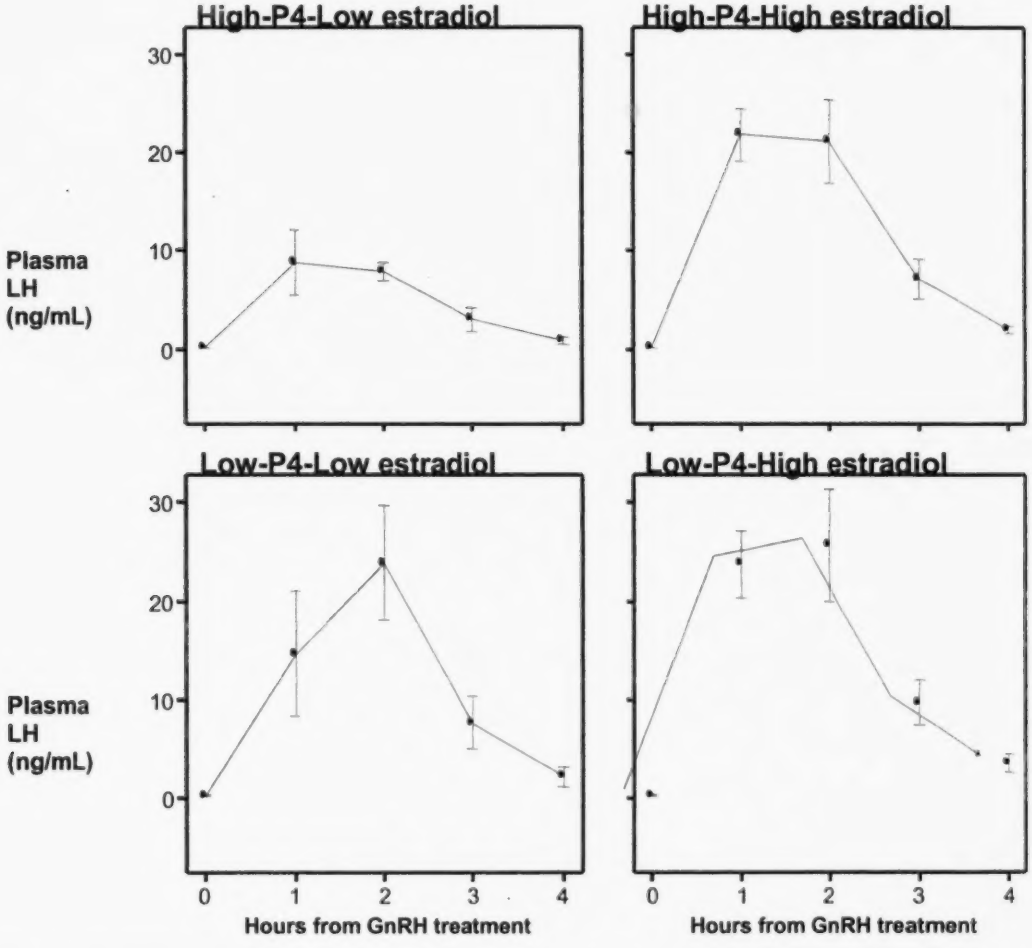

Figure 9. Mean (±SEM) plasma concentrations of luteinizing hormone (LH) in heifers with high (High-P4) or low (Low P4) circulating progesterone concentrations and treated with or without 0.25 mg estradiol benzoate 12 h prior to 100 μg GnRH. Luteinizing hormone concentrations 1 h after the administration of GnRH were higher in heifers given estradiol than those not given estradiol 12 h prior to GnRH

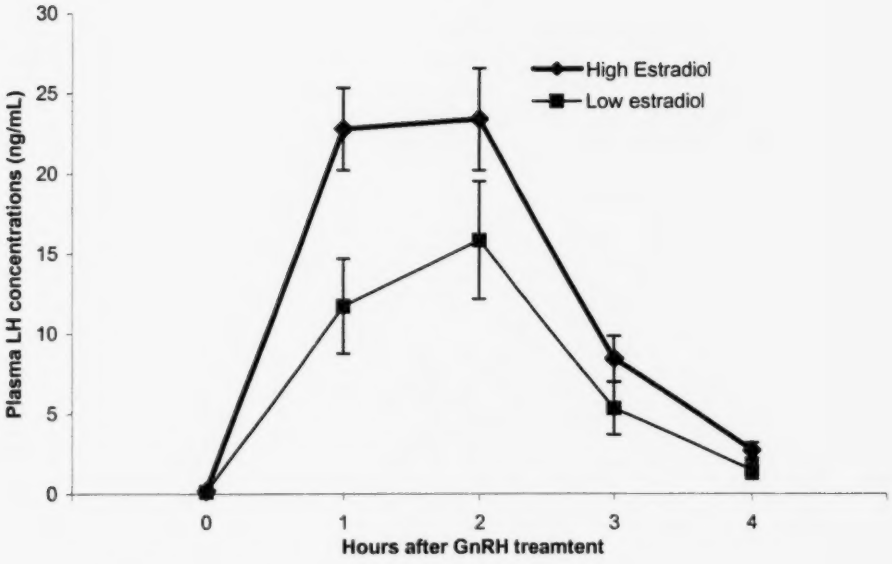

Figure 10. Mean (±SEM) plasma concentrations of luteinizing hormone (LH) following the administration of 100 μg GnRH in heifers that had or had not been pretreated with 0.25 mg estradiol benzoate 12 h earlier, regardless of progesterone concentrations. Pretreatment with estradiol overcame the suppressive effects of elevated concentrations of progesterone on LH release but did not improve ovulation rate.

In summary, results tended to confirm the effects of blood progesterone concentrations on LH release in response to an injection of GnRH; this seemed to be overcome by the administration of estradiol, suggesting that the LH-deprived follicle may have produced sufficient estradiol to induce LH receptors in the pituitary gland. However, pretreatment with estradiol did not improve ovulation rate, suggesting that there may have been an effect on the follicle

Overall, there was no difference between heifers and lactating beef cows; the latter appeared to be more similar to heifers than lactating dairy cows. Progesterone concentrations also affected preovulatory follicle diameter making it difficult to measure effects on ovulatory response. Increasing the dose of GnRH did not increase LH release while pretreatment with estradiol did; neither increased ovulatory response. Results have important implications for the use of GnRH-based TAI protocols in heifers. At present, the only alternative is to add a progesterone-releasing intravaginal insert between the first injection of GnRH or pLH and the injection of PGF in order to achieve acceptable pregnancy rates to TAI in heifers.

CONCLUSIONS AND RECOMMENDATIONS

The overall objective of this proposal was to refine protocols that synchronize ovulation resulting in high fertility to timed-AI (TAI; AI by appointment), without the need for estrus detection. Specific objectives were to simplify protocols, use commercial products, and improve pregnancy rates to TAI by increasing the proportion of cattle responding to follicle synchronizing treatments and promoting growth and maturation of the preovulatory follicle. Specific observations are as follows:

1) Although estradiol benzoate treatment 7 days after a single injection n of PGF effectively synchronized follicular development, progesterone treatment at that time appeared to suppress CL function. Results do not provide convincing support for the use of estradiol and progesterone in a PGF-based TAI protocol.

2) Although MGA could offer a reasonable alternative as a progestin source for a TAI protocol in beef heifers, clearance of MGA from the digestive tract appears to create issues relative to timing of ovulation. On the other hand, a 7-day or 8-day CIDR protocol was very effective.

3) MGA appeared to be useful for presynchronization of heifers.

4) Presynchronization with PGF or a used CIDR plus PGF increased the numbers of animals responding to the first injection of GnRH or pLH in a GnRH-PGF-GnRH protocol for TAI; effects on pregnancy rates were variable and not conclusive.

5) Although the addition of a progesterone-releasing vaginal insert to a GnRH-based protocol resulted in increased pregnancy rates in heifers, there was no benefit in lactating beef cows.

6) Presynchronization showed no benefit in estradiol-CIDR based protocols.

7) Treatment with eCG at the time of PGF treatment increased pregnancy rates in the younger cows whether they were synchronized with GnRH or estradiol; there was no benefit in heifers.

8) The administration of eCG at the predicted time of follicle wave emergence resulted in higher pregnancy rates in recipients submitted to timed-embryo transfer.

9) Treatments designed to increase post-TAI progesterone levels did not increase pregnancy rates to TAI or embryo transfer, but post-TAI progestin treatment synchronized returns to estrus.

10) The CL forming from a persistent follicle was as efficacious as that from normal preovulatory follicle in supporting pregnancy in embryo transfer recipients, but it did not produce more progesterone.

11) The addition of a CIDR or eCG to a GnRH-based synchronization protocol yielded no improvement in recipient selection rates, or pregnancy rate following fixed-time embryo transfer.

12) Elevated levels of progesterone suppressed GnRH-induced LH release and ovulation. However, there was no difference between heifers and lactating beef cows, which appeared to be more similar to heifers in response to GnRH treatment than lactating dairy cows.

13) Progesterone concentrations affected preovulatory follicle diameter making it difficult to measure effects of progesterone on GnRH-induced LH release and ovulatory response.

14) Increasing the dose of GnRH did not increase LH release while pretreatment with estradiol did; neither increased ovulatory response.

15) Results have important implications for the use of GnRH-based TAI protocols in heifers.

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Personnel

Reuben J. Mapletoft – Investigator (University of Saskatchewan)
John P. Kastelic – Co-investigator (AAFC, Lethbridge)
Julie Small – Collaborator (AAFC, Brandon)
Jimena Yapura – Graduate Student
Fernanda Diaz – Graduate Student
Marcos Colazo – Graduate student/Post-doctoral Fellow
Marcelo Martinez – Graduate student/Post-doctoral Fellow
Mary Rutledge – Summer student
Hannah Davis – Summer student
Nathan Erickson – Summer Student

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